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The Network Structure of Elastin in the Grain Layer of Cattle Hide*

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ABSTRACT

The recent isolation of a condensed layer of elastin from the grain surface of a cattle hide appeared contrary to the histological findings which indicated fine fibers distributed generally throughout the grain layer. By horizontal sectioning and chemical fractionation of the sections the condensed layer of elastin was shown to be the result of the collapse of a three-dimensional network of elastin which extends throughout the thickness of the grain layer. A maximum concentration of elastin occurs at about one-third the depth of the grain layer. Commercial trypsin was able to dissolve this elastin network from the surface layers.

INTRODUCTION

The properties of the grain surface of leather are different from those of a split surface because of a different architecture in the grain surface. It can be shown histologically (1, 6) that the grain side of a cattle hide as prepared for tanning has a surface layer which is composed of much finer fibers than are found in the lower layers of the hide. This layer also contains the functional structures of the hide such as hair follicles, erector pili muscles, sweat glands, and sebaceous glands, which are not present in the lower hide layers. Different workers have described all or part of this top layer by the terms "grain layer", "papillary layer", "thermostat layer", and "Corium minor".

As the collagen fibers of this upper portion are continuous with those of the lower portions of the hide, any plane of demarcation between the two layers is necessarily an arbitrary one. Some workers (Roddy, private communication) take the plane of the sweat glands as the lower boundary of the grain layer. For purposes of this report, "grain layer" will be used to designate the region of the hide from the grain surface to a plane slightly below the bases of the deepest hair follicles.

Recently (2) a dense, filmy layer was separated from the grain surface of a limed cattle hide. Visual observation led to the belief that this film actually came from the top surface of the hide and it was, therefore, called the grain membrane. The properties of this material indicated that it was elastin. Histological studies (1, 6), however, show that the elastic fibers of the hide

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are fine fibers intimately interwoven with the collagenous and reticular fibers. They are more numerous in the grain layer and are very prominent around the hair follicles.

In order to determine the location of the supposedly dense grain membrane within the grain layer, stratigraphic analysis of the grain layer was attempted. Kritzinger and Theis (3) have used this method to study the distribution of chromic oxide in leather. They, however, divided the entire thickness of the hide into only ten layers. For the present work the grain layer alone was cut into fifteen layers. These layers were analyzed by a method similar to that of Lowry, Gilligan, and Katersky (4), except that the thin slices of tissue were not ground before analysis, the fractions were assayed by a Kjeldahl nitrogen determination instead of weighing, the autoclaving was done at 23 pounds steam pressure, and the elastin residue was not extracted with boiling alkali before analysis. Extraction with boiling alkali was omitted so that our residue would correspond more nearly to the grain membrane we were trying to locate. This membrane was obtained by autoclaving at neutrality, and Neuman and Logan (5) have shown that a residue obtained in this way may contain small amounts of unidentified substances which are not elastin.

EXPERIMENTAL

All the material studied was taken from a single large bull hide which was obtained freshly flayed and washed with running water for one-half hour in a slotted drum to remove blood and debris. After fleshing, this hide was stored at -20° F. until used. For each run a piece about four inches square taken from the bend area was thawed and then immersed in one liter of 2.5% sodium cloride solution at room temperature. This solution contained 0.15g. of phenylmercuric acetate as a preservative. After 48 hours the hair and epidermis were loose enough to be pulled off by hand. The few short hairs that remained were pulled out with tweezers. The surface was then scudded with a spatula to squeeze any loose material from the hair follicles. The piece was cut into approximately 1-cm. squares which were kept in fresh salt solution until sectioned.

The squares were frozen grain side down on the stage of a freezing microtome and the flesh side was shaved off until the center of the corium was reached. Two slices each 0.1 mm. thick were cut from this corium region. The analysis of these slices is reported as slice 18 in Table I. The piece was then thawed just enough to free it from the microtome stage. The grain side was placed on top and the piece again frozen to the microtome stage. The microtome stage was adjusted so that the entire grain surface was even with the microtome blade. For most pieces the surface was sufficiently flat that the first slice (0.1 mm. thick) could be cut across the entire area of the piece. If a complete cut was not obtained by the second slice the piece was rejected.

TABLE I

ELASTIN AND TOTAL NITROGEN CONTENT OF GRAIN SLICES*

	Run 1		Run 2		Run 3		Trypsin-treated	
Slice	Elastin	Total	Elastin	Total	Elastin	Total	Elastin	Total
1	1	1314	20	803	21	1370	0	564
2	29	1744	41	1078	24	1275	0	853
3	60	1673	28	1314	53	1298	8	938
4	60	2202	24	1449	40	1418	11	1174
5	81	2554	57	1594	83	1568	19	1250
6	91	2789	41	1712	55	1451	51	1497
7	40	1600	65	2005	53	1588	45	1446
8	62	2710	35	1738	60	1766	42	1624
9	33	2400	32	1827	70	1941	50	1641
10	64	2107	60	1903	34	1994	58	1837
11	56	2507	39	2043	28	2090	61	1808
12	58	2410	38	2111	25	2284	59	1888
13	39	2400	36	2088	31	2828	62	1876
14	41	2448	30	2059	19	2218	59	1964
15	17	2441	38	2143	17	2772	30	2060
16	14	2542	31	2047	25	2642	25	1793
17	31	2512	26	1890	20	2905	16	1868
18†	23	5132	13	4143	9	6823	36	6959

*Kjeldahl nitrogen titer as 100 x ml. of 0.02 *N* acid.
†Composite of 2 slices from center of corium.

The first slice can be expected to be slightly thinner than the others because of inability to set the microtome blade exactly at the surface of the hide. The subsequent slices were all cut 0.1 mm. thick. The possible error in the position of any part of a slice will, therefore, be in the neighborhood of 0.1 mm.

Immediately after cutting, each slice was put into a 15-ml. conical centrifuge tube numbered to correspond to the slice number. Seventeen slices were taken from each piece. The last hair follicle, which indicates the lower boundary of the grain layer, disppeared at about the fifteenth slice. A total of six or seven pieces were cut to make up the sample for each run Thus each run represented a section through 6 or 7 sq. cm. of hide.

The tissue slices in each tube were extracted at room temperature with 2 ml. of 0.1 *N* sodium hydroxide for 16 hours. The tubes were centrifuged and the liquid was decanted into a semimicro Kjeldahl digesting tube. The residue was resuspended in fresh alkali. After one hour the tube was again centrifuged, and the second extract was added to the first. The residue was suspended in 2 ml. of water, neutralized, and centrifuged. This wash was added to the corresponding alkali extracts. The residue was suspended in 2 ml. of water and autoclaved for 2 hours at 23 pounds steam pressure. After cooling and centrifuging, the gelatin extract was decanted into a semi-

micro Kjeldahl digesting tube. The residue was resuspended in 2 ml. of water and reautoclaved for another hour. This second gelatin extract was added to the first. The residue was washed into another semimicro Kjeldahl digesting tube.

The extracts and the residue were digested with 2 ml. of sulfuric acid to which 1.20 g. of potassium sulfate and 0.03 g. of mercuric oxide were added. The ammonia liberated from the digestion mixture was caught in boric acid solution and titrated with acid. The number of milliliters of 0.2 *N* acid required for the elastin residues and the sum for the two extracts and the residue are recorded in Table I.

One piece of the same bull hide dehaired as previously described was suspended in a 0.2% solution of commercial trypsin buffered at pH 9.0 and incubated for 3 hours at 37° C. After the treatment the piece was washed thoroughly with water and allowed to stand overnight in water with phenylmercuric acetate as a preservative. The trypsin-treated material was sectioned and analyzed as described above. The results are recorded in Table I.

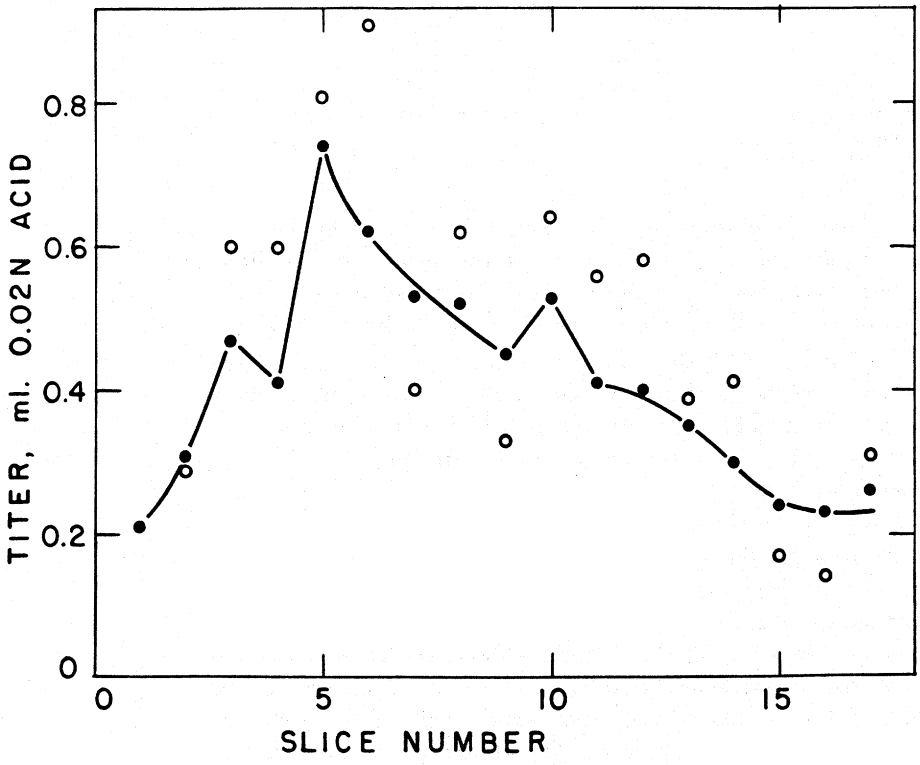

FIGURE 1.—Elastin nitrogen content of serial slices from bull hide grain layer
● Average for 3 runs
○ Values for run 1

DISCUSSION

The swollen nature of the hide pieces at the time they were cut introduces several variations which must be considered before the data of Table I can be satisfactorily interpreted. No attempt was made to cut the sample pieces to reproducible size or dimensions for these would be meaningless unless the moisture content of the pieces could be accurately controlled. This would be very difficult for such highly swollen material. The area of tissue cut was, therefore, slightly different for each run and might even be different for each slice in a run if the edges of the piece were not cut exactly perpendicular to the surface of the piece. This latter variation should be slight for the angle of deviation from the perpendicular was not noticeably great.

Variation in moisture content is not limited to differences in the degree of swelling of the material cut for the various runs, but may also occur between the individual slices within a run because the composition and structure of the grain layer are not uniform throughout its thickness. The small amount of material obtained from each slice was not sufficient to give a reliable dry weight. Histological studies indicate that the grain layer is predominately collagen; therefore, the nitrogen content was chosen as an index of the quantity of material present in the various slices.

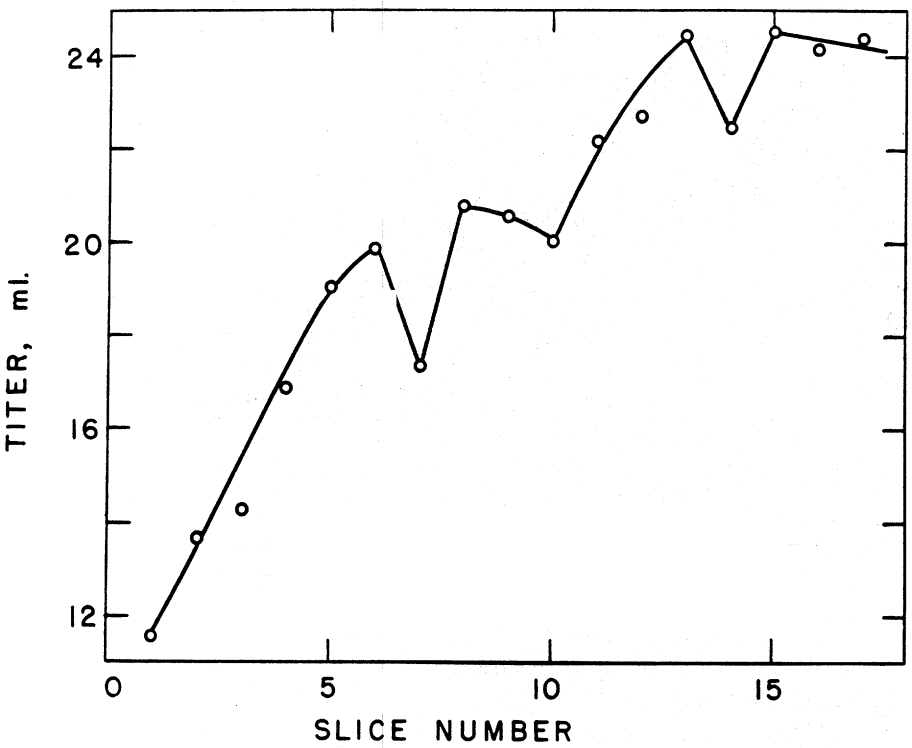

FIGURE 2.—Average total nitrogen content of serial slices from bull hide grain layer

The average value for the elastin nitrogen content of the various slices for the first three runs is shown in Figure 1. The individual data of the first run are also presented to show the variations encountered in a single run. The oscillations between high and low values shown by this run in the region between the fourth and eleventh slices also occur in the other two runs. This may be an indication of the existence of strata of higher and lower elastin contents which are not shown by the average curve. However, all the peaks and valleys cannot be superimposed by shifting the curves along the slice number axis. More closely controlled experiments will be necessary to clarify this detail.

The average value of the total nitrogen content for the slices from the first three runs is plotted in Figure 2. The gradual rise in this value as the distance from the surface increases indicates that the surface layers of the hide are either high in moisture content, contain a high proportion of non nitrogenous material, or are full of voids left by the removal of materials from the hair follicles during the dehairing treatment. When the elastin nitrogen for

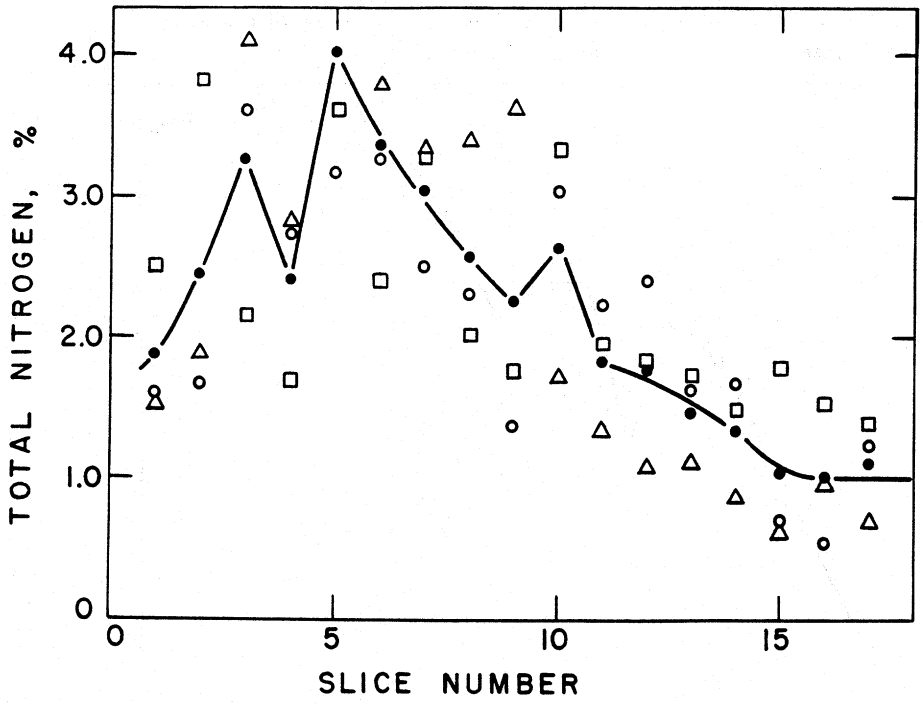

FIGURE 3.—Elastin nitrogen as percent of total nitrogen

● Average for 3 runs
○ Values for run 1
□ Values for run 2
△ Values for run 3

each slice of each run is divided by the total nitrogen value for that slice the ratio obtained should be independent of all constitutional variations which do not affect the ratio of the nitrogenous constituents of the hide. The values for this ratio are presented in Figure 3 along with the average value and a smoothed curve drawn through the average data where conditions permit. The oscillations between high and low values noted in the untreated data have been modified to some extent by this calculation, but they have not been completely eliminated. It is quite possible that these variations are due partly to stratification of the elastin and partly to the presence of strata or localized concentrations of nonnitrogenous constituents such as mucopolysaccharides and lipids. The low points in the total nitrogen values which occur at about slices 6, 7, and 8 for the different runs are probably caused by the large fat concentration in these slices. These slices correspond to the region where the sebaceous glands should be found. The extracts for these slices show the effects of high fat concentrations.

These variations in the individual values, however significant they may prove to be, should not be allowed to detract from the main observation

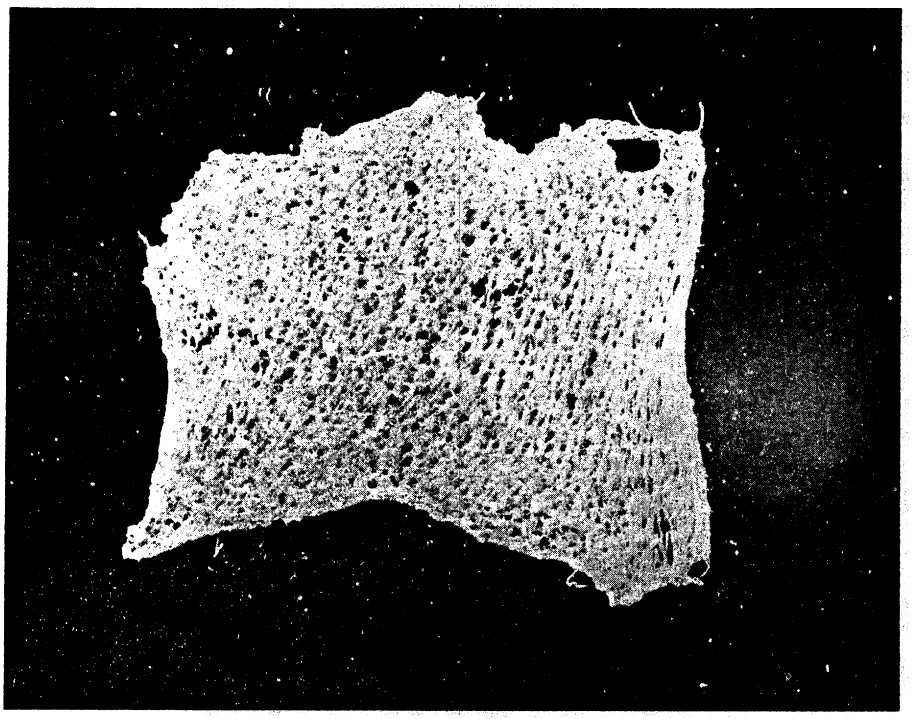

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FIGURE 4.—Elastin network from slice number 5. Dimensions at centers of the sides are 5.0 mm. wide and 7.0 mm. long.

that the elastin material does not appear to be concentrated in any dense layer near the surface of the hide. It is distributed generally throughout the entire grain layer. Its concentration appears to increase to a maximum value at about one-third the depth of the grain layer and then to recede to lower concentrations as it approaches the corium. The ratio of the elastin to total nitrogen at the point of maximum concentration in the grain layer is approximately thirteen times the ratio obtained for slices taken from the center of the corium.

One of the remarkable characteristics of the elastin residues obtained from most of the slices was their retention of a film-like structure. The elastin fibers appear to be anastomosed into a three-dimensional network which is shown in Figure 4. The film-like structure of the elastin residue persists to about the fifteenth slice, which is just about at the depth of the deepest hair follicle. The residue obtained from slice number 17 is shown

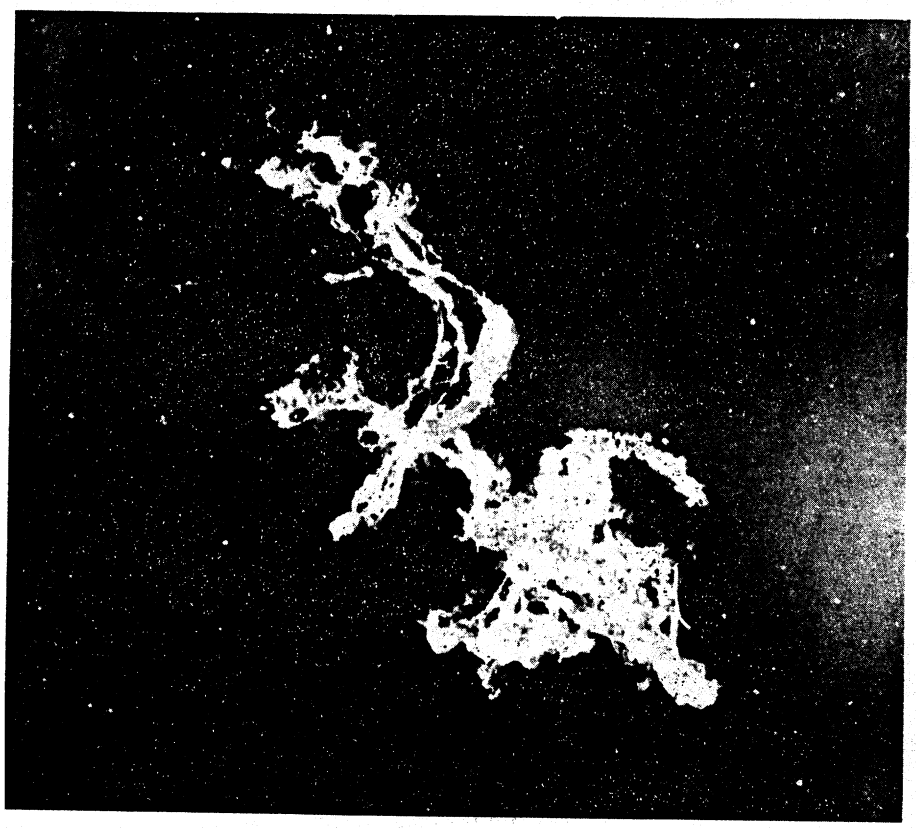

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FIGURE 5.—Elastin network from slice number 17. Longest dimension is 7.5 mm.

in Figure 5. There are a few connected fibers but the structure is mostly disintegrated.

The dense elastin layer–grain membrane which was previously isolated (3) appears to be the result of a collapse of the three-dimensional network demonstrated here. This can be illustrated by suspending the freshly isolated undehydrated grain membrane in a gelatin or agar solution and allowing the gel to solidify. A cross section of the embedded grain membrane shows a thickness of approximately 1.5 mm. which is the thickness of the original grain layer from which it was made.

Commercial trypsin has been reported to digest elastin from hide materials, and the effect of a three-hour treatment with a 0.2% trypsin solution at 37°C. on a piece of our raw hide material has been studied by the stratigraphic technique. The results are reported in Table I, and the ratio of the elastin nitrogen to total nitrogen is plotted in Figure 6 with the average curve from Figure 3 as a comparison. The complete removal of the elastin from the two surface slices and the partial removal of the elastin from the next three slices are demonstrated. The elastin content of slices 6 through 10, and 15

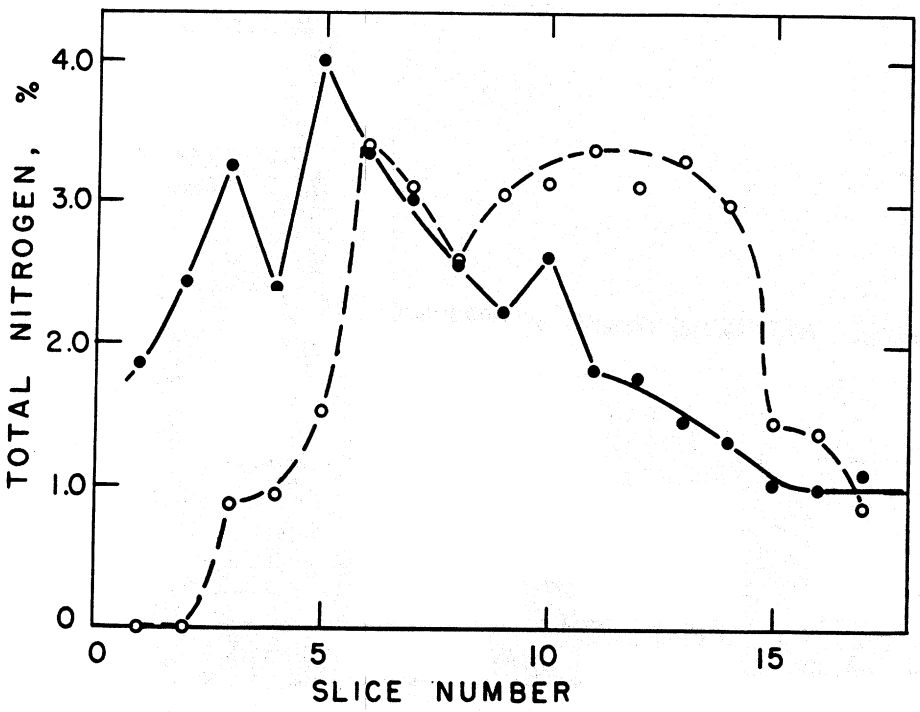
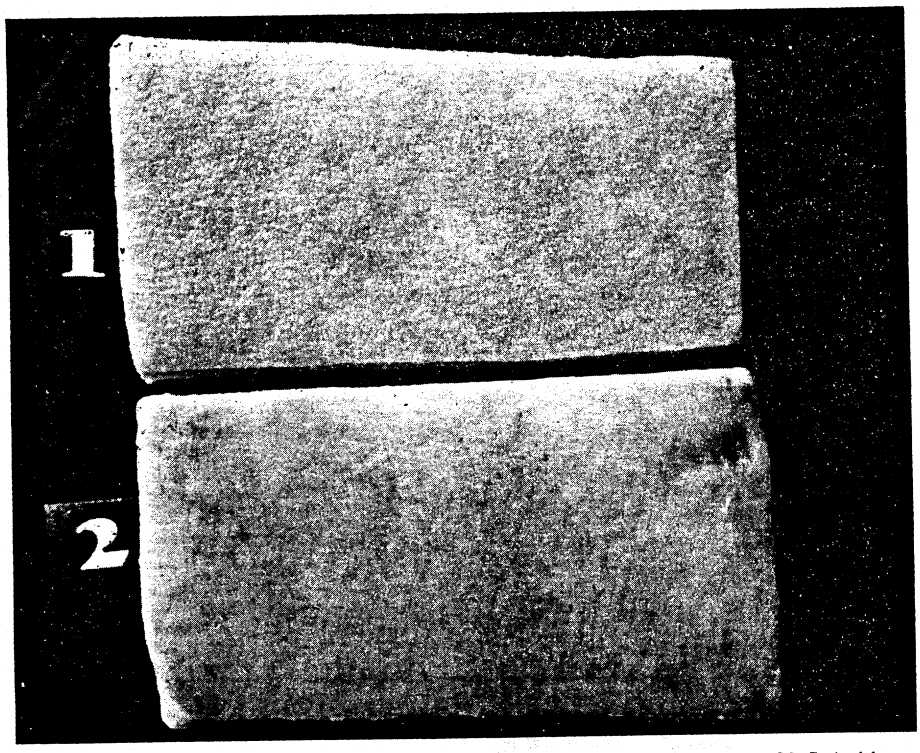


FIGURE 6.—Elastin content of trypsin-treated bull hide grain layer

● Average of untreated hide
○ Trypsin-treated hide

through 17, falls in the range of the untreated samples. The high values for slices 11 through 14 cannot be explained.

These results indicate that, although commercial trypsin will digest the elastin material of a hide, the rate of its penetration into the hide is very slow. In this experiment the penetration was only 0.5 mm. in three hours. The effect of this slight penetration upon the surface characteristics of the hide pieces is remarkable. Figure 7 shows the changes in the grain of sample 2 which was trypsin-treated compared with the full grain of sample 1 which received the same treatment except for the enzyme. The surface layer of the trypsin-treated hide is somewhat transparent, and the dark residues in the hair follicles have become visible. The elasticity of this sample was also impaired, for the forceps marks which rapidly disappeared from the grain of the control piece remained in the enzyme-treated piece for a considerable time, as can be observed in the upper right corner of piece Number 2.



Courtesy of U. S. D. A. Photo by M. C. Audsley.

FIGURE 7.—Surface changes produced by treating bull hide with trypsin: 1, control; 2, trypsin-treated.

CONCLUSION

The results show that the elastin of the grain layer is distributed in a three-dimensional network which extends from the grain surface to the bottom of the grain layer. The density of the network rises slightly from the surface layers to a maximum at about one-third the depth of the grain layer and then recedes to low levels as the corium is approached. The dense elastin membrane previously isolated is formed when the three-dimensional network collapses on itself.

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